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Laboratory Evaluation of Marine Fishes as Carcinogen Assay Subjects

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In regard to this role, there is a need for practical, experimental exposure systems for the short term, and long term exposure of fishes and invertebrates in order to evaluate their responses to environmentally significant carcinogens. We have designed and tested and adaptable, aquatic laboratory system for flowing water or static water assays of carcinogenic or suspect carcinogenic agents against marine fishes. We repor here the design, results of long term tests, and the future uses of the system for determining the risks of carcinogenic agents in the aquatic environment, and as a syst compementary to mammalian assay systems, but which permits the phylogenetic expansion of carcinogen assay methodology.

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Laboratory Evaluation of Marine Fishes as Carcinogen Assay Subjects

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Abstract: The U.S. Environmental Protection Agency (EPA) and the National Cancer Institute (NCI) have major responsibilities for determining the fate and risks of carcinogenic agents in the natural environment. Under the auspices of EPA/NCI, the Carcinogen Research Team at the U.S.E.P.A. Laboratory, Gulf Breeze, has a major role in investigating the fate, effects, and risks of carcinogenic agents in the aquatic portion of the biosphere.

In regard to this role, there is a need for practical, experimental exposure systems for the short term, and long term exposure of fishes and invertebrates in order to evaluate their responses to environmentally significant carcinogens. We have designed and tested an adaptable, aquatic laboratory system for flowing water or static water assays of carcinogenic or suspect carcinogenic agents against marine fishes. We report here the design, results of long term tests, and the future uses of the system for determining the risks of carcinogenic agents in the aquatic environment, and as a system complementary to mammalian assay systems, but which permits the phylogenetic expansion of carcinogen assay methodology.

A pilot test of the described system has been completed. This test utilized flowing filtered, estuarine water, controlled water temperature, controlled photoperiod, controlled nutrition of test species, oxygen concentration monitoring, and various life cycle stages of the test fish, the sheepshead minnow, *Cyprinodon variegatus*, and the suspect carcinogenic herbicide, trifluralin. Continuous exposures to 1 to 5 μg/l trifluralin were conducted with zygote, through embryogenesis to adult stages of the sheepshead minnow over 19 months.

Results revealed that the system maintained the control and experimental fish successfully for 19 months, during which abnormal growths appeared on the vertebral column of the trifluralin exposed fish, both in those exposed from zygote to adult and in those exposed only as adults. Control fish presented no lesions. The assay specimens, and control specimens were studied utilizing the techniques of analytical chemistry, biochemistry, physiology, histopathology, radiology, and electron microscopy. The vertebral lesion is characterized as a bony dysplasia, and not

as a real neoplasm. The system, however, has proven to be very adequate and versatile, for the exposure of marine fish, and assessment of induced lesions.

Future uses of this system will involve the assay of both chemical and physical agents in either flow through or static modes. More species of marine fishes and invertebrates will be tested for various life cycle stage responses to suspect carcinogenic agents in order to identify sensitive indicator and model species with biochemical, physiological, cellular, and histological methods.

The role of comparative oncology and comparative pathology in the phylogenetic approach to understanding the evolution, and hence, the genesis of cancer has been and will be discussed at this symposium by several speakers. Perhaps our best chance for understanding the complex phenomenon of neoplasia lies in the study of diverse species with similar neoplasms, as well as in studying similar species with similar and diverse neoplasms.

In our work we have come to perceive that the central problem of extrapolating the significance of observation from one phyletic level to another is to recognize where similarities in biology end, and differences begin. Figure 1 attempts, in a nonquantitative, arbitrary manner to depict the biologic commonness and differences among several phyletic levels in regard to biology. Biologic commonage, particularly at certain levels (Fig. 1), may outweigh the biologic differences among animal species from different classes and phyla. On the other hand, biologic differences at other levels (Fig. 1) may be so great as to nullify certain comparisons or conclusions drawn from superficial observations on unrelated or distantly related

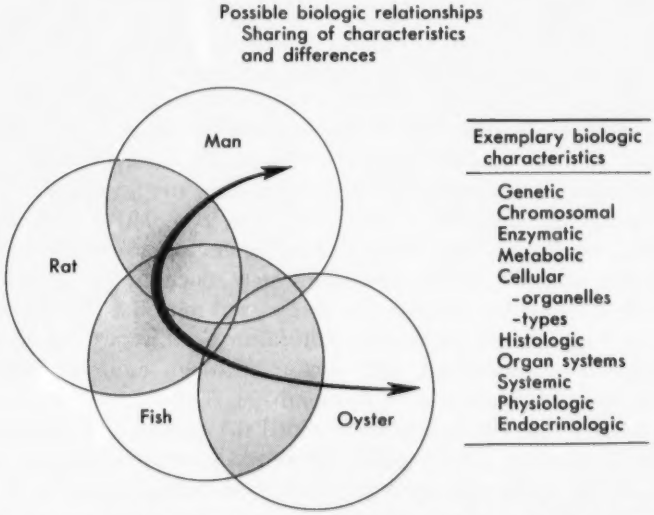


FIG. 1. An analogy of circles to suggest non-quantitative phyletic relationships among several unrelated species of interest to cancer researchers. Shaded areas of circles indicate shared or closely related biologic characteristics; open areas in circles indicate non-shared characteristics of features (uniqueness).

species. Most probably the validity of drawn comparisons will be substantiated only by the specific knowledge and insight of the individual investigator for the systems being compared. Simply stated: the more we know about the systems compared, the more valid our conclusions (probably) should be.

With these concepts as our points of departure, we have undertaken the development, and testing of a laboratory carcinogen assay system for marine and estuarine fishes. The need for practical laboratory test systems, utilizing marine fishes, is generated by the fact that coastal and estuarine areas are increasingly the recipients of chemical pollutants, and by the relatively recent findings that tumors of various kinds have been found at unusually high frequencies in certain coastal fish and shellfish populations. Therefore, it is highly desirable to know as much as possible about the responses of marine fishes to known or suspect carcinogens under rigidly controlled laboratory conditions.

Criteria for the system, under our development, were selected to make the system as flexible and useful as possible, yet to permit control of certain environmental factors (Fig. 2). The following optional uses and control characteristics were considered in our design: 1) flowing sea water and static sea water or freshwater maintenance of organisms for long periods; 2) control of salinity, light intensity and period, and water temperature; 3) control of nutritional status of test organisms; and 4) successful maintenance of zygote through adult stages of fishes tested if desired (complete life cycle, single life stage exposure).

Our system, to date, has utilized the sheepshead minnow (*Cyprinodon variegatus*) and the Gulf killifish (*Fundulus grandis*). These two species, both in the family Cyprinodontidae, may be considered the "mouse" and "rat," respectively, of this assay system (Fig. 3). In the present paper, we will present the results of the first

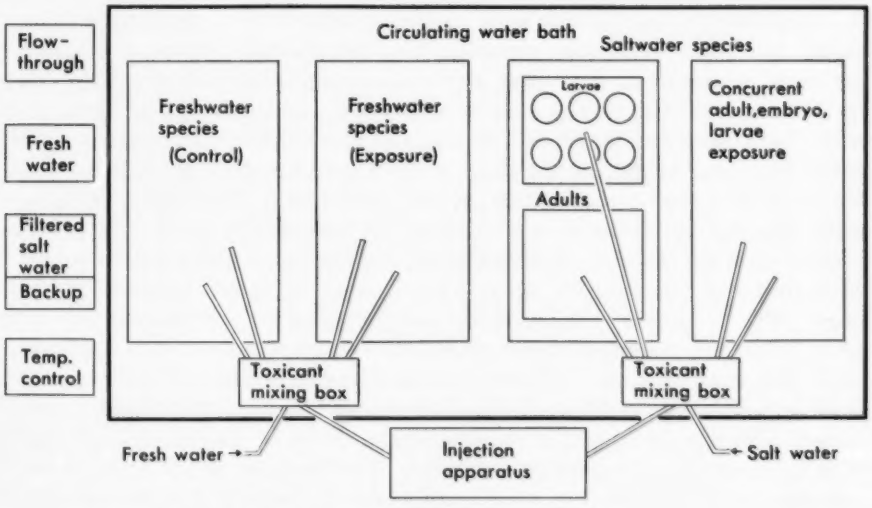


FIG. 2. General schematic of fish carcinogen assay system illustrating the optional uses and application of the system and its organization.

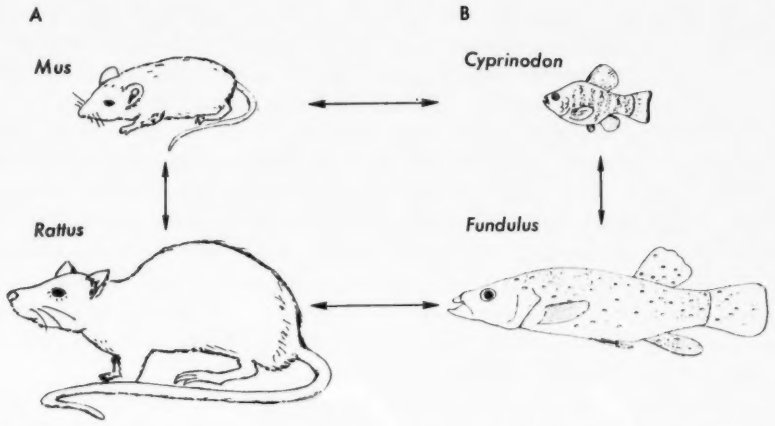


FIG. 3. Aquatic species presently used as test or assay specimens in Gulf Breeze system. Relationships between *Cyprinodon* and *Fundulus* may be analogous to relationships between *Mus* and *Rattus*. (A) Standard mammalian test animals. Family-Muridae. (B) Aquatic surrogates. Family-Cyprinodontidae.

pilot, long-term test of one of these fish, the sheepshead minnow, against a suspect carcinogenic chemical, the herbicide trifluralin.

MATERIALS AND METHODS

1. *Aquatic animal carcinogen assay system*

Schematic Figs. 4–7 illustrate the design of the laboratory assay system.

The aquatic carcinogen assay system designed consists of three basic sections: the water preparation complex, the water/toxicant delivery complex, and the exposure complex. The water preparation complex consists of a large reservoir (preheating head box in Fig. 4) located above the testing area. Sea water, pumped from Santa Rosa Sound, Florida at our laboratory and filtered through a series of sand filters, was chosen as the main water source for the test, with fixed salinity sea water in a reserve reservoir in case of pump failure. The option exists here for using fixed salinity as the main water source if desired. The water is supplied to the preheating head box on a demand basis; float switches and solenoid valves (Fig. 4) control and maintain the water level. A 5,000 W quartz enclosed submersible heater (Fig. 4) located in the reservoir and controlled by a thermostat with a sensor probe located in the water is used to maintain water temperature at approximately 30°C. Vigorous aeration is provided in the reservoir to prevent temperature stratification and maintain dissolved oxygen levels within acceptable limits. A dissolved oxygen sensor probe is used to monitor oxygen levels as desired with the capability of using a recorder for constant monitoring. The final part of the water preparation complex is a 5 μm filter (Fig. 4) through which the water is pumped enroute to the working head box, the first part of the water delivery complex.

Water level in the working head box (Fig. 4) is controlled by a float switch

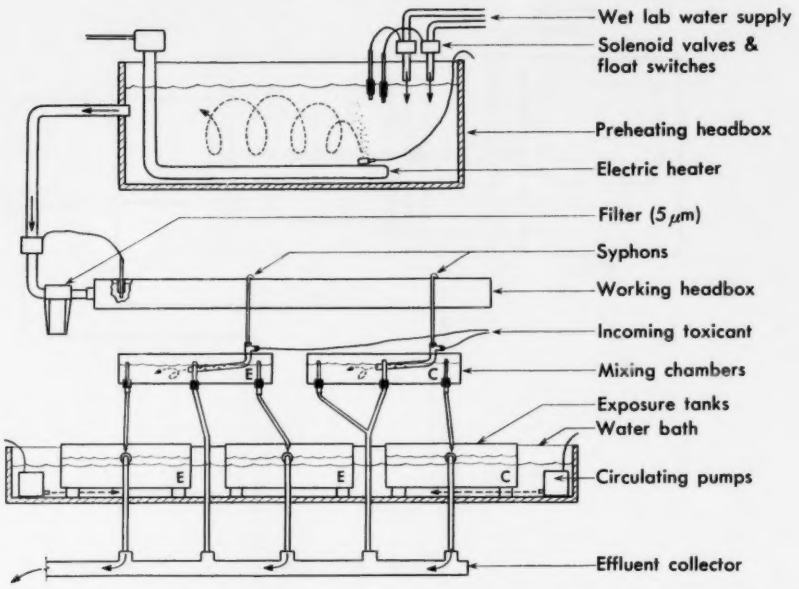


FIG. 4. Diagram of carcinogen assay system showing major and minor components and their general relationship. E, experimental chambers through which toxicant flows; C, control situation.

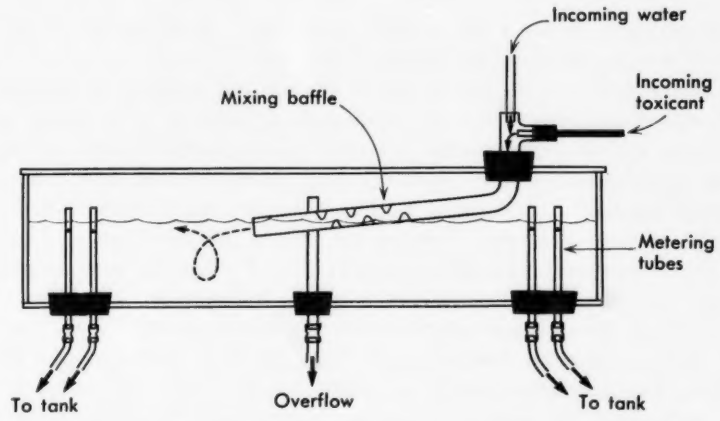


FIG. 5. Diagram of mixing chamber. Toxicant (or carrier) and incoming sea water (of calibrated flow) are mixed, then distributed to exposure tanks *via* metering tubes.

and solenoid valve which provides water on a demand-only basis. This conserves water and lessens demand on the pumping and filtering facilities. Water is fed from the working head box to the second part of the water delivery complex, the mixing chambers (Figs. 4 and 5), *via* glass syphon tubes (Fig. 4) calibrated to a given flow

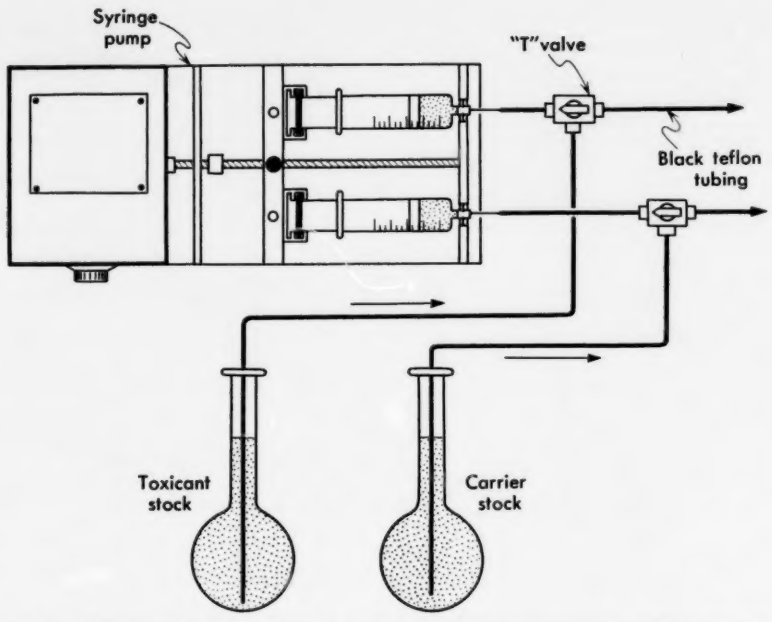


FIG. 6. Diagram of injection apparatus with "T" valve arrangement to refill syringes. Provides rapid recharge of syringes and minimal potential for operator contact with toxicant.

rate. Introduction of the toxicant and distribution of the water to the exposure complex is accomplished in the mixing chambers.

Toxicant stock (a mixture of solvent carrier and toxicant) is metered into the flow of water emerging from the calibrated syphons (Fig. 5) using a Harvard infusion/withdrawal syringe pump (Fig. 6). This metering system can be operated continuously or intermittently. Teflon tubing, painted black externally to prevent exposure of the toxicant to light, is used to make all connections between the syringe pump and mixing chambers. T-valves are placed in these lines and tubing is extended from the valves to stock containers (Fig. 6). During normal operation the valves are set so that fluid flow runs from the syringes to the mixing chambers. When stocks in the syringes are expended the valves are turned so that when the syringe pump is reversed fluid is drawn from the stock reservoirs and the syringes are refilled. This procedure minimizes operator contact with toxicants and facilitates syringe refill. After the toxicant enters the water flow, the water/toxicant mixture then cascades through the mixing baffle (Fig. 5) and passes on into the mixing chamber proper. For control purposes, pure carrier if used, is metered into the water entering the control mixing chamber in a similar fashion.

Water exits the mixing chambers *via* metering tubes (Fig. 5). These consist of 3 mm inside diameter (ID) tubing with a 1.5 mm diameter hole located 20 mm from the top. Flow rates emerging from the metering tubes are adjusted by varying the position of the hole in the water column. Calibration of the tubes is accomplished

as follows: the initial tube is adjusted to give the desired flow rate using a stop watch and measuring the amount of water delivered in a given period. A pattern of air spaces is established in the out-flowing water within the tube. Using an adjustable strobe light, the air/water pattern is "frozen," thus a calibration setting is obtained from the strobe corresponding to the desired flow. Using this strobe setting all the metering tubes are adjusted to a uniform flow.

Four metering tubes exit each of the mixing chambers; in addition, an overflow (Fig. 5) exits each chamber and is connected to an effluent collector tube (Fig. 4). Glass tubing is used to effect connection of the metering tubes to the appropriate positions in the exposure tanks (Fig. 4).

The exposure complex consists of a series of three glass aquaria (Fig. 4), a control and two exposure aquaria (receiving water from a single mixing chamber). The aquaria are located in a fiberglass water bath (Fig. 4) containing a thermostatically controlled 5,000 W titanium sheath submersible heater to maintain a

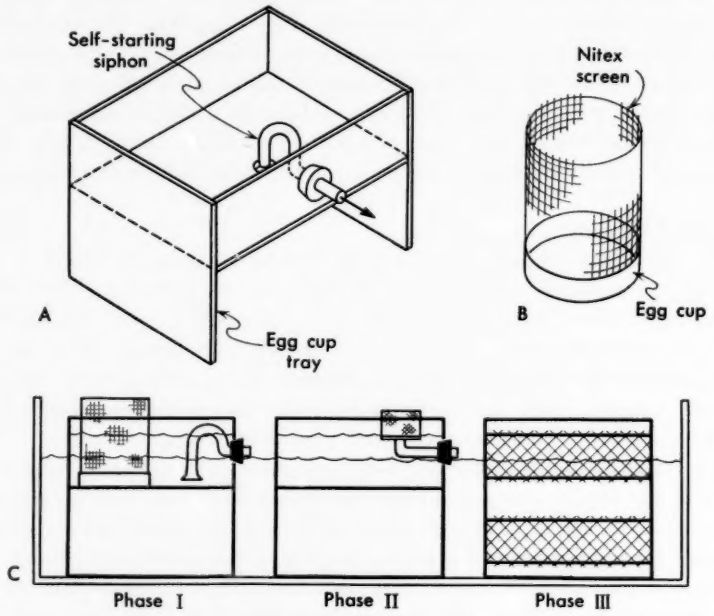


FIG. 7. (A) Diagram of egg cup tray, constructed of glass, equipped with self-starting syphon. (B) Diagram of egg cup used within egg cup tray. Several may be used in each tray to provide an efficient method of holding and handling fish zygotes and young fry. (C) Diagram depicting various methods of holding fish during test. Phase I, egg cup tray containing egg cups, represents arrangement used during zygote to early fry stages. Self-starting syphon provides fluctuation of water level to insure flushing of water within each cup and hence, uniform exposure of test organisms to toxicant. Phase II, egg cup tray modified with screen protected stand-pipe, providing holding area for fry and juvenile fish. Allows use of various life stages simultaneously (*i.e.*, fry and juveniles within tray, adults in large outer aquaria). Phase III, holding cage within larger aquaria, for adult fish when juveniles are released into outer aquaria from Phase II situation.

temperature of approximately 30°C and two submersible pumps (Fig. 4) to maintain circulation of the water for even heat distribution. Overflow water is shunted *via* glass tubing to an effluent collector pipe (Fig. 4) which empties into a drainage system leading to a settling/treatment pond.

The test system is designed for flexibility. The basic system, with minor modifications could be used to test a wide variety of aquatic organisms, freshwater or marine. The system, as constructed, is capable of testing various stages in the life cycle of a fish (*i.e.*, zygote, embryo, fry, juvenile, and adult) with a minimum of modification, and also, to test different stages simultaneously. This is accomplished as follows: located within the exposure aquaria are removable egg cup trays (Fig. 7A) (*1*) consisting of shallow glass trays with end pieces extending downward as legs. They are designed so that the sides of the trays will be above the water level in the large exposure aquaria. The trays can either be fitted with self-starting syphons (Figs. 7A and 7C, Phase I) so that the water level within the tray will fluctuate approximately 3 cm, or with screen-protected overflow tubes (Fig. 7C, Phase II) providing a constant water level. Egg cups (Fig. 7B) consisting of 9 cm internal diameter glass petri dish bottoms with 450 μm nylon mesh collars attached, can be placed in the egg cup trays for easy handling of developing eggs and young fry. Glass holding cages (Fig. 7C, Phase III) with screen bottoms and side pieces can also be used within the large aquaria for holding larger fishes. The specific application for each of these accessories will be elaborated in the next section.

2. Specific pilot study

The pilot study for the assay system described above involved a chronic exposure of *C. variegatus*, the sheepshead minnow, to trifluralin (2,6 dinitro-N,N-dipropyl-4-(trifluoromethyl) benzamine), a commonly used pre-emergent herbicide. The trifluralin stock (Eli Lilly Lot #X-29959, Treflan) was prepared with a concentration of 654.5 mg/l using triethylene glycol as a carrier. This stock concentration coupled with an injection rate of 0.275 ml/hr entering an incoming water flow of 30 l/hr produces a nominal concentration of 6.0 μg/l.

Our test was designed for exposing organisms through all stages of their life cycles and for testing various stages simultaneously. The testing scheme was as follows: adult *C. variegatus* were collected from Santa Rosa Sound, Florida, and acclimated for a minimum of 2 weeks in holding tanks similar to those to be used for testing. Acclimating water was supplied from the same source as testing water, temperature maintained at 30°C and a photo-period of 12 hr light and 12 hr dark established. Twenty-five of these fish were placed in each of the three large exposure aquaria. One tank served as a control and received water containing carrier only. The remaining two tanks were for experimental exposure (designated as E1 and E2). Tank E1 received water from the experimental mixing chamber containing trifluralin at 1 to 5 μg/l (measured). Tank E2 received water from the control mixing chamber for the initial phase of the test.

Each of the three large exposure aquaria also initially contained 2 egg cup trays, fitted with self-starting syphons (Fig. 7C, Phase I), containing 6 egg cups each. Incoming water entered the test systems from the mixing chambers in two

streams of approximately 7.5 l/hr each (15 l/hr per exposure aquaria), one stream entering each of the egg cup trays. Water and toxicant ran through the test system for a week prior to beginning the test to allow for an equilibrium to be established.

Three days prior to beginning the test, 10 acclimated adult female *C. variegatus* were injected intraperitoneally with 50 international units (IU) of human chorionic gonadotropin (HCG) (*2*). The following day each fish was again injected with 50 IU of HCG. HCG is used as an agent to induce maturation of eggs present in gravid females. This insured the harvest of a large number of mature viable eggs at a predetermined time. On the day the test began, the 10 females were sacrificed, the eggs removed and placed in filtered sea water at the temperature and salinity of test conditions. The testes from 10 acclimated male *C. variegatus* were macerated in a few ml of sea water. The sperm suspension was added to the eggs and gently mixed. After 1 hr, the water was decanted and replaced with fresh sea water. The fertilized eggs were then separated from non-fertilized ones and placed in a separate container.

Twenty-five fertilized eggs (Fig. 8) were placed in each egg cup (Fig. 7C, Phase I), a total of 300 eggs for each of the three large aquaria (one control and two experimental). Zygote development was observed by removing an egg cup from the system and placing it under a dissecting scope: mortality of zygotes and yolk fry were also observed. Fry were fed newly-hatched brine shrimp twice daily. At

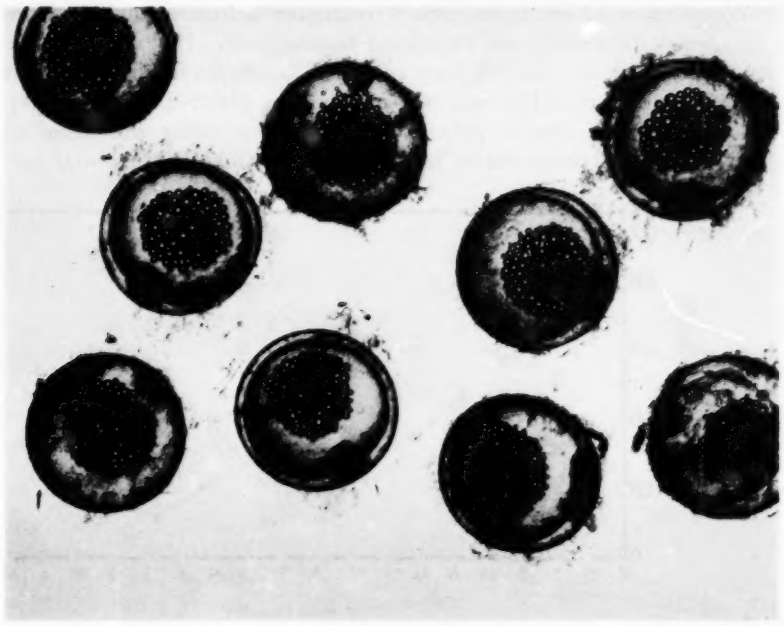


FIG. 8. Eggs at time of introduction into test system, approximately 2 hr post-fertilization (four cell stage). × 18.

30 days post-fertilization, the water source for the experimental (E2) tank was changed from the control mixing chamber to the exposure mixing chamber.

At 40 days post-fertilization, the juvenile fish were removed from the egg cups and placed within the egg cup trays (Fig. 7C, Phase II), which had the self-starting syphons replaced with screen-protected over-flow tubes. As the fish increased in size, feeding was supplemented with commercially prepared dry flake food and frozen adult brine shrimp.

Approximately 4 months after the test began, the glass holding cages were placed into the test system, one in each aquarium (Fig. 7C, Phase III). The adult fish were captured and placed within the holding cage. The test-reared fish were then released into the large aquaria and the egg cup trays removed from the system. This was the arrangement for the duration of the test.

3. Sampling and processing

The assay system was operational for a total of 19 months. During this period periodic water samples were taken for gas chromatographic chemical analyses (performed by chemical support facilities at the Environmental Protection Agency (EPA) laboratory, Gulf Breeze, Florida). Figure 9 plots concentration (μg/l) *vs.* time (months) for the testing period.

Monitoring of the developing fish was performed daily throughout the test. During the first 220 days samples were taken for histology on a monthly basis. The first sample (day 30) consisted of 5 fish from each test situation, the remaining samples consisted of 10 fish from each. Cumulative sampling totaled 225 fish, control and exposed, processed and examined histologically. Histological processing was preformed as follows: the fish were fixed in Davidson's fixative, the visceral cavity of each fish was opened to insure rapid fixative penetration. They were then dehydrated and embedded in paraffin. The fish were either embedded whole or, as fish size increased, were cut in half on the longitudinal horizontal median plane

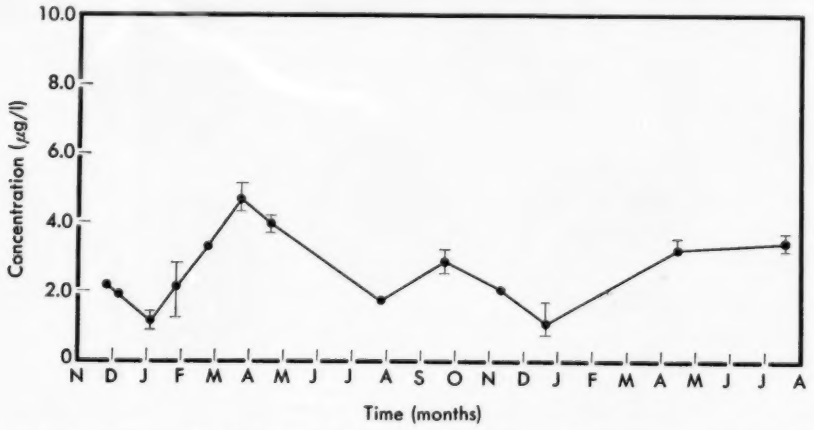


FIG. 9. Concentration of trifluralin in water column during 19 month study. Data points represent average concentration (μg/l) of water samples taken, with range for each datum.

and both halves embedded in a single block. Enough serial sections (longitudinal horizontal) were cut from each fish to ensure good representative sections of the vertebrae. Sections (5 to 7 μm) were stained with Harris hematoxylyn and eosin and examined microscopically.

Pathologic examination was also performed using radiological sampling. Beginning at day 251 and approximately every 3 months until test-termination, samples of 10 fish from each test situation were taken for radiological examination. (Radiographs of several previous histological samples had been taken and had shown us the reliability of the radiological examination in diagnosis of the vertebral lesion which we are reporting). The procedure for our radiologic samples follows: fish were selected randomly in groups of 10. Using 150 mg/l tricaine (MS-222) in sea water, the fish were anesthetized to the point at which operculum movement ceased (approximately 2.0 to 2.5 min). The fish were then removed from the water/anesthetic solution, lightly dried and placed on a sheet of Kodak M-2 (Ready Pak) X-ray film. X-rays were made using a Faxitron 43805N X-ray system (exposure of 0.45 min at 50 KVP). The fish were then returned to clean sea water, permitted to revive, and observed, before being returned to the assay system. X-rays were developed using Kodak D-19 developer for 5 min.

The test was terminated on day 583, at this point, all fish (those started as adults as well as those started as zygotes) remaining in the assay system (119 in all) were removed and X-rayed. Necropsies were performed on each fish. Several fish (5) were selected for vertebral processing, using radiographs as a guide. Trunk sections containing the radiograph diagnosed lesions were removed and processed histologically. The brain, visceral mass and body trunk of all fish were preserved for future study.

RESULTS

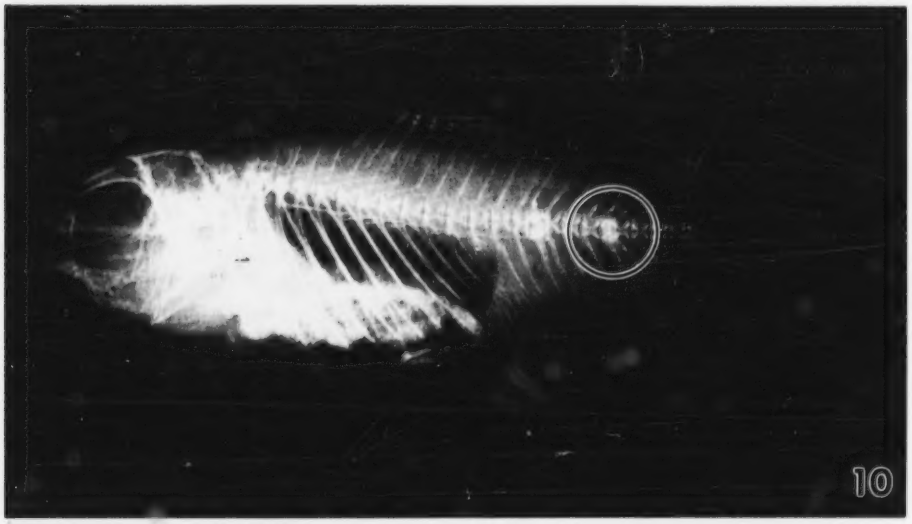
1. *Efficacy of assay system for long term tests*

The described system has maintained control and experimental sheepshead minnows for different experimental purposes very satisfactorily for 19 months.

TABLE 1. Frequencies of Trifluralin Induced Vertebral Lesions in Sheepshead Minnows (*Cyprinodon variegatus*) Exposed for 19 Months

Test animal (*C. variegatus*)	Vertebral dysplasia[a]	No. of fish with one lesion[b]	No. of fish with two or more lesions[b]	Total No. with lesions[b]
Control	0	0	0	0
(E1) Exposure started at fertilization (zygote)	26/31 (83.9%)	5/31 (16.1%)	5/31 (16.1%)	10/31 (32.2%)
(E2) Exposure started at days past fertilization	34/39 (87.2%)	17/39 (43.6%)	4/39 (10.3%)	21/39 (53.8%)
(E1A) Exposure started with adults	3/7 (43%)	0	0	0

[a] Diffuse dysplasia: enlargement and malformation of vertebrae. [b] Lesion: focal hyperostosis.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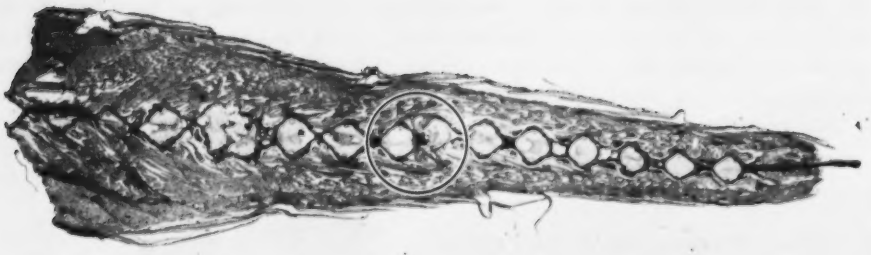
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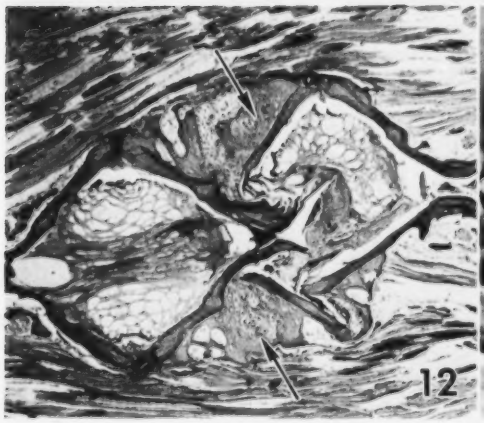
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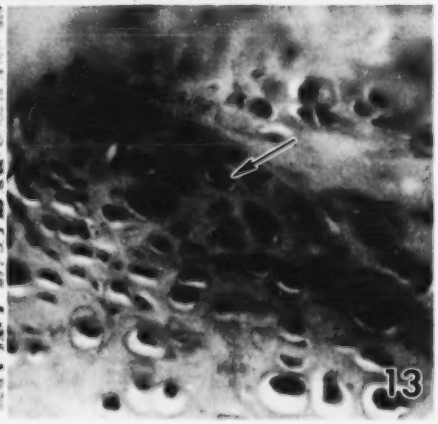
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During the study described in the preceding section, ambient water exposure concentrations of the test chemical, trifluralin, were maintained within a relatively narrow range of from 1 to 5 μg/l for the entire test (Fig. 9). This indicates that the injection apparatus and water delivery system functioned adequately in maintaining a reasonable exposure of test fish to desired concentration of test chemical.

Control, non-exposed fish that were reared from fertilized egg (zygote) to adults during the course of the 19 months exposure period developed normally. Lesions encountered in exposed fish were related to effects of the test chemical and not to conditions inherent in the assay system.

In the absence of a test chemical, the Gulf killifish (*F. grandis*) was reared from zygote to young adults in the system. This indicates that, at least, other marine species related to the sheepshead minnow can be maintained successfully in the system for experimental manipulation of their early life cycle stages.

2. Trifluralin associated, vertebral lesions in exposed Cyprinodon

No neoplasms were found in *Cyprinodon* exposed in the 19 month test. Radiographic and histologic examination, however, revealed a high incidence of diffuse vertebral dysplasia and focal vertebral lesions (Table 1). In fish exposed for their entire life (E1, Table 1) over 83% had radiographic and histologic signs of diffuse dysplasia of vertebrae. Over 87% of fish exposed from 30 days post fertilization (E2, Table 1) to 19 months had signs of diffuse vertebral dysplasia. Forty-three percent of fish exposed as adults only (E1A, Table 1) had the signs of diffuse vertebral dysplasia. This dysplasia resembled that described by Couch *et al.* (*3*) in *Cyprinodon* exposed in earlier test to trifluralin. (see below)

Focal hyperostosis (Fig. 10) constituted the second kind of vertebral lesions associated with trifluralin exposure. Only those fish exposed in early life stages (E1, Table 1) or as young adults (E2, Table 1) had this type lesion. From 32 to 35% of these fish had one or more focal lesions in their vertebral columns (Table 1; Figs. 10–13). The radiographic and histologic characteristics of this focal hyperostosis should be briefly described.

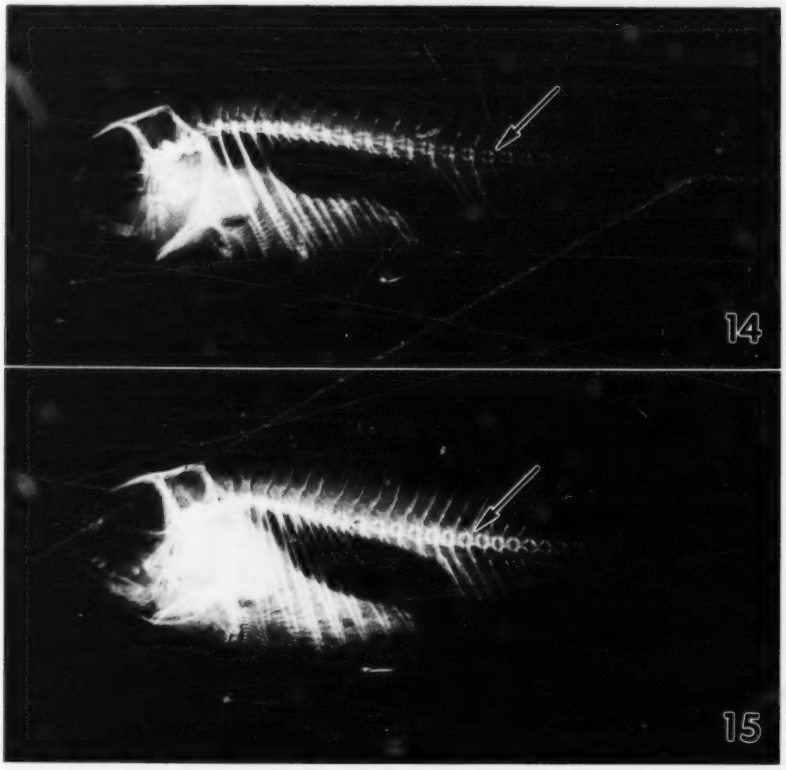
In previous studies (*3*), we described a diffuse dysplasia, characterized as a general hyperostosis of the vertebral column, in *Cyprinodon* exposed to higher concentrations of trifluralin for shorter periods. The focal lesions reported here for *Cyprinodon* exposed to lower concentrations (1–5 μg/l of trifluralin) share most of the histologic and cytologic characteristics of the previously reported diffuse dysplasia with the exception of being limited in size. As we have mentioned, high numbers of all fish exposed in this test also had the diffuse dysplasia (Figs. 15–16).

←FIG. 10. Radiograph of *Cyprinodon* exposed for 19 months to 1 to 5 μg/l trifluralin. This specimen grew from zygote to young adult in the trifluralin. Note the focal hyperostosis (circle), one of two vertebral lesions in this fish. Compare to normal *Cyprinodon* in Fig. 14. ×3.4.

←FIG. 11. Longitudinal, horizontal section of fish in Fig. 10. Note the focal lesion revealed histologically, that is the same as circled in Fig. 10. ×9.75.

←FIG. 12. Higher magnification of focal lesion illustrated in Figs. 10 and 11. Note the masses (arrows) of new bony tissue surrounding damaged vertebrae. ×55.

←FIG. 13. Cells, probably osteoblasts, in bony tissue of focal lesions. ×517.



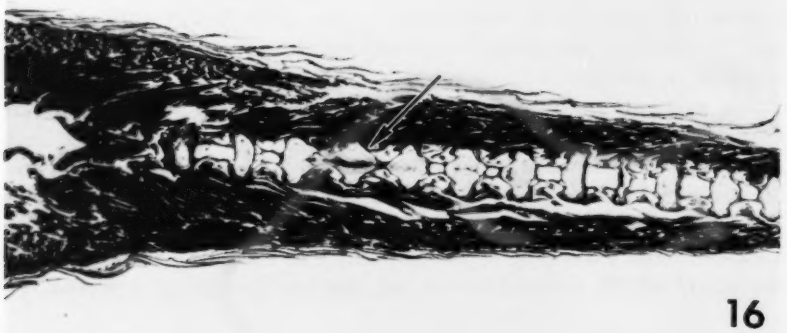


FIG. 14. Radiograph of normal *Cyprinodon*. Note the low opacity of normal vertebrae (arrow). ×2.3.

FIG. 15. Radiograph of *Cyprinodon* exposed for 19 months to 1–5 μg/l trifluralin. Note the greater opacity and density of vertebrae (arrow) in dysplastic vertebral column. ×2.1.

FIG. 16. Longitudinal horizontal section of diffusely dysplastic vertebral column as found in fish in Fig. 15. Arrow indicates dysplastic vertebra. ×11.

Figures 10 to 13, are, however, presented to demonstrate the limited and asymetrical characteristics of the focal lesion induced in fish exposed for 19 months (Table 1). This focal hyperostosis is apparently related to osteoblastic or fibroblastic cells (Figs. 12 and 13) that have been stimulated directly or indirectly to focally produce bone and bone precursors in the fish that were exposed in their early life stages. The lesion is not neoplastic, and may merely represent bony repair tissue or attempts to rebuild or stabilize vertebrae damaged by exposure to trifluralin in early growth or development.

It is pertinent to point out that none of the control fish maintained during the course of the test developed any vertebral lesions or other signs of disease. These included those control fish reared from zygote to adult in the control segment of the system during the entire 19 months of the test.

DISCUSSION

Marine fishes, as well as more domesticated freshwater species, can be utilized as test organisms for carcinogen assays. A practical assay system for this purpose must have the capability of sustaining fish species selected in a healthy but artificial background environment in order to minimize extraneous stress as a factor in the test.

Marine fishes have been found to suffer from many tumor types found in freshwater fishes and other vertebrates including mammals. Certain tumors have been found in relatively high frequencies in coastal populations. We have designed an assay system that should be adaptable to most coastal laboratories. Variations of the preceding system could also be used in static modes with artificial sea water or with freshwater fishes.

The system described herein will be used in the future at Gulf Breeze with a variety of suspect carcinogenic agents and several species of fishes and invertebrates.

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